

# A lipoxxygenase is the main lipid body protein in cucumber and soybean cotyledons during the stage of triglyceride mobilization

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The 90-kDa lipid body protein characterized earlier by its high expression during the stage of fat degradation was identified as a form of lipoxxygenase. This organelle form was compared with lipoxxygenase species purified from the cytosol. It is further shown that the antibodies raised against the lipid body membrane lipoxxygenase from cucumber cotyledons cross-react with both cytosolic and lipid body lipoxxygenase from soybean.

*Cucumis sativus; Glycine max; Lipid body; Lipoxxygenase*

## 1. INTRODUCTION

Nonadienal and other volatile C<sub>9</sub> compounds are characteristic compounds of cucumber fruits and seedlings [1–3]. Their biosynthesis was proposed to take place by cleavage of 9-hydroperoxi-octadecadienoic acid which results from the action of lipoxxygenase on linoleate [4]. However, it is likely that within the family of lipoxxygenases enzymes may be included which act on esterified polyenoic fatty acids [5,6], e.g. membrane constituents or lipoproteins [7]. It was proposed that vertebrate 15-lipoxxygenases may oxygenate the fatty acid moiety of cholesterol esters and may thus change the role of LDLs [8].

We report here, for the stage of maximal fat degradation in plant cells, the identity between a well-described constituent of the lipid body membrane [9] and the predominant form of lipoxxygenase. The co-existence of triglycerides with linoleoyl residues and lipoxxygenase within a cell compartment implies that lipoxxygenase plays a physiological role in triglyceride metabolism in lipid bodies.

## 2. MATERIALS AND METHODS

Cotyledons of 4-day-old cucumber or soybean seedlings were used as enzyme source. Lipid body fractions were prepared according to [9]. Antiserum was raised against the 90-kDa protein isolated from purified lipid bodies of cucumber cotyledons [9]. Fragments of the 90-kDa protein were prepared by exhaustive tryptic cleavage at pH 7. Peptides were isolated by two consecutive chromatographies on reversed phase material at pH 2.5 and pH 7, respectively. N-terminal sequences were determined by Edman degradation in a gas-phase sequencer.

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The cytosolic species of the 90-kDa protein was isolated from a 200,000 × g supernatant by fractionation with ammonium sulfate and by chromatography on DEAE-cellulose and subsequent hydrophobic-interaction chromatography on Fractogel TSK-butyl. Lipoxxygenase activity was assayed according to [10] using linoleate or trilinolein as substrate.

## 3. RESULTS

### 3.1. The 90-kDa protein of lipid bodies at the stage of maximal fat mobilization is a lipoxxygenase

Purified 90-kDa protein from cucumber lipid bodies was subjected to trypsin cleavage, and the amino acid sequences of three peptides were compared with sequences in the EMBL database. It showed homology between the 90-kDa protein and plant lipoxxygenases characterized by a size of approximately 95 kDa (Fig. 1).

Our search for a cytosolic precursor form of the lipid body protein resulted in the detection of soluble 90-kDa species which cross-reacted with the antibodies raised against the lipid body 90-kDa protein (Fig. 2). Following chromatographic purification of the cytosolic enzymes, three forms of lipoxxygenase differing in size (Fig. 2) and charge (data not shown) were characterized. The purification of the soluble 90-kDa forms led to the isolation of protein with the specific lipoxxygenase activity of 600 nkat/mg protein.

### 3.2. The germination-related forms of lipoxxygenase is also found in lipid bodies of soybean

As most previous work on plant lipoxxygenases has been done with extracts from dry seeds or plants of soybean [11,12], we investigated whether a lipoxxygenase is located in the lipid bodies of cotyledons of this plant. Using the polyclonal antibodies raised against the cucumber lipoxxygenase we identified, in cotyledons of ger-

	Amino acid sequence	residues	identity (%)
90 kDa soybean	--YFEEELWNL	178-189	78
3 Pea	K-YFEEELHNLR	186-195	78
	H-YFEEELNNL		
90 kDa soybean	--LYIVGFHIALMPY	423-436	46
Pea	R-LFLLGHHDFIMFY	431-444	39
	K-LFLLDHSDSIMFY		
90 kDa soybean	--LFPYNLPYTYAVP	827-841	39
Pea	R-CGFVQMFYTLLEP	835-849	39
	R-HGPVEMFYTLLEP		

Fig. 1. Alignment of amino acid sequences of peptides obtained by fragmentation of the 90-kDa lipid body protein and of soybean lipoxygenase-3 [20] and pea lipoxygenase [21]. The N-termini of the fragments are preceded, in the sequences compared, by lysine or arginine.

minating seeds, lipoxygenase in the cytosol and in the purified lipid body fraction (Fig. 3). Similar data were obtained for melon seedlings (data not shown).

#### 4. DISCUSSION

Lipid bodies which are formed during seed development [13,14] receive a new set of proteins [9] during germination, i.e. during fat mobilization. The most highly expressed species among these newly synthesized proteins is the 90-kDa protein, now identified as lipoxygenase. A possible model supposes that lipid bodies constitute the compartment where lipid degradation starts, not only by the formation of odorant aldehyds representing the characteristic smell of cucumber fruits, but mainly by yielding a  $C_9$  acyl moiety. Subsequent to the formation of the 9-hydroperoxi derivative of linoleoyl groups, cleavage may yield a  $C_9$  compound originating from C-atoms 10-18 of the unsaturated fatty

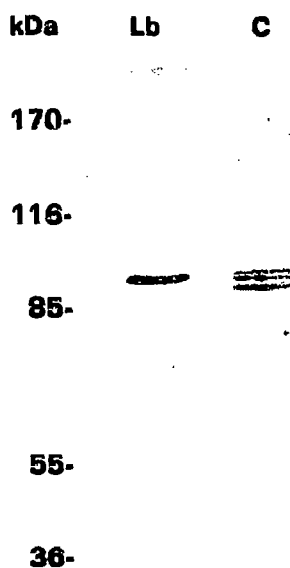


Fig. 2. Denaturing electrophoresis of lipoxygenase preparations and immunoblotting with antibodies raised against the 90-kDa protein. Lipid body membrane proteins (Lb); purified cytosolic lipoxygenase of cucumber cotyledons (C).

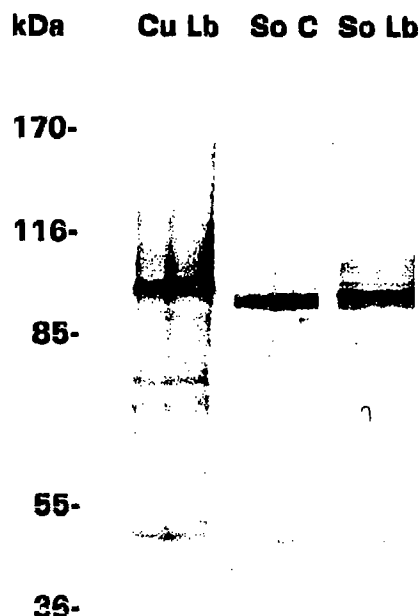


Fig. 3. Occurrence in soybean lipid bodies of a 90-kDa protein immunologically related to the cucumber 90-kDa protein. Samples of lipid body proteins from cucumber cotyledons (Cu Lb) or soybean cotyledons (So Lb), and from soybean cytosol (So C) were separated by denaturing electrophoresis, blotted on nitrocellulose and immunodecorated with antibodies against the cucumber 90-kDa protein.

acid and another  $C_9$  compound bound as triglyceride intermediate. Thus, an oxidative modification of triglycerides, including the hydroperoxide formation, may precede the hydrolysis leading to fatty acids and their further degradation on glyoxysomes [15,16]. Together with several pathways leading to jasmonate [17] and  $C_{18}$  oxoderivatives, a number of metabolic sequences may start out by the action of lipoxygenases [4]. Our search for a pathway of triglyceride mobilization not initiated by lipase activity resulted from the fact that cucumber and soybean lipid bodies lack lipase activities ([15]; Kindl, unpublished results).

Irrespective of a lipoxygenase function in triglyceride metabolism, lipoxygenase is implied in the  $O_2$ -dependent elicitation of a signal transduction chain which acts in response to the attack by a pathogen [18].

We included lipid bodies from soybean where most work with plant lipoxygenases has been done. It became evident that a lipoxygenase similar to the form occurring in various cucurbitaceae is also localized on soybean lipid bodies. Earlier immunochemical investigations with soybean cotyledons and excluded such a possibility [19].

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